

# LEVELS OF CONFIDENCE IN THE ANALYSIS OF HYBRIDIZATION IN PLANTS<sup>1</sup>

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## CRITERIA OF HYBRIDIZATION

One of the hypotheses often used to explain many present-day variation patterns in plants is that they result from interspecific hybridization followed by stabilization of hybrid derivatives. To test this hypothesis we make use of several criteria. Our level of confidence depends on the adequacy of the criteria and the extent to which they are satisfied. This, in turn, depends on various features of the biology of the plants under study. For example, several criteria are employed at the polyploid level which cannot be applied at the diploid level. These criteria have to do with chromosome number, chromosome pairing in experimental inter-ploidal hybrids, tetrasomic inheritance, and the colchicine-synthesis of artificial polyploids from F<sub>1</sub> hybrids between the progenitors. The use of these additional criteria provides a stronger test of hybridity at the polyploid level than can be made at the diploid level.

At the diploid level, the number of criteria are fewer, and they are equivocal. As a result it is more difficult to recognize stabilized diploid hybrid entities, and the literature contains only a small number of examples relative to the number of examples of allopolyploids. The putative diploid hybrids include Corn Belt Dent corns (Anderson & Brown, 1952), *Potentilla glandulosa* subsp. *hansenii* (Clausen, Keck & Hiesey, 1940), *Achilea rosea-alba* (Ehrendorfer, 1959), several subspecies of *Gilia capitata* (Grant, 1950), *Gilia achilleaefolia* (Grant, 1954), *Gilia ochroleuca* subsp. *vivida* and *G. cana* (Grant & Grant, 1960), *Phlox maculata* subsp. *pyramidalis* (Levin, 1966; Hadley & Levin, 1969), *Phlox pilosa* subsp. *deamii* and *P. amoena* subsp. *lighthipei* (Levin & Smith, 1966), *Delphinium gypsophilum* (Lewis & Epling, 1959), *Clarkia deflexa* (Lewis & Lewis, 1955), *Lasthenia ferrisiae* (Ornduff, 1966), the "Abbeville Red" Iris (Randolph, Nelson & Plaisted, 1967), *Penstemon spectabilis* and *P. clevelandii* (Straw, 1955), and *Purshia glandulosa* (Stutz & Thomas, 1964).

The primary criterion of hybridity is morphological intermediacy in several characters; in fact, in the absence of this condition, a hybrid origin would not be suspected. Other criteria include:

- 1) An additive profile for biochemical characters such as flavanoids or seed proteins that are present in each parent but not in both of them.
- 2) Unusual amounts of interpopulation morphological variability presumably resulting from continued segregation of parental differences.
- 3) Distribution in the zone of geographical overlap of the parents.
- 4) Occurrence in more recent geological formations than the parents.

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5) Occurrence in ecologically intermediate habitats and intermediacy for physiological characters.

6) Existence of at least partial fertility in  $F_1$  hybrids between the parents to permit the possibility of the production of segregant genotypes.

7) Experimental synthesis of individuals resembling the hybrid taxon in segregants of hybrids between the parents (at least partially achieved by Ehrendorfer, 1959; Grant, 1950; Lewis & Epling, 1959; Ornduff, 1966; Stebbins, 1957).

With the exception of experimental synthesis, none of these criteria are strong, because they do not rigorously distinguish between the hypothesis of interspecific hybridization and alternative hypotheses. Thus, morphological intermediacy and biochemical additivity may reflect ancestral and not derivative status, or selection of mutants that occurred independently in the gene pool rather than having been introduced by hybridization. An unusual amount of inter-populational morphological variability may reflect differences in breeding system, environmental heterogeneity, or founder effect. The occurrence of the taxon on recent or ecologically intermediate formations may also indicate the result of selection of genetic variants already present in the gene pool. Partial fertility of  $F_1$  hybrids does not provide information about the likelihood of forming  $F_2$  and backcross progeny and whether they would survive in nature. Where experimental studies have been done, for example, in *Gilia* (Grant, 1966), most segregating hybrid progeny were inviable and highly sterile and were kept alive only by the most careful greenhouse practices. Consequently, we see that the individual criteria do not provide a rigorous and unequivocal means of testing the hypothesis that a particular diploid entity has a hybrid origin. The criteria should be more accurately considered a series of questions; the more of them that are answered positively, the higher is the level of confidence that our hypothesis is correct.

#### INTRODUCTION TO *STEPHANOMERIA*

I would like to illustrate the problems of using these criteria by examining the variation patterns and reproductive relationships of the annual species of the western North American genus *Stephanomeria*, a member of the Chicory tribe of the Compositae. The annual *Stephanomerias* are closely related species widely distributed in a number of habitats in California and adjacent regions. They have long been known for their complex morphological intergradation which has stymied taxonomic efforts to separate the taxa one from another (Ferris, 1960: 574). They constitute both a polyploid complex and a homogamic complex in which the same divergent diploid species are connected by morphologically intermediate tetraploid and diploid taxa. They are now known to include nine diploid and two tetraploid entities (Gottlieb, 1969; unpublished). A formal taxonomic classification of the annual *Stephanomerias* has not yet been set forth, because certain additional field studies are required before taxonomic rank can be determined. Eight of the diploid taxa are divided into two distinct clusters on the basis of morphology, cytology, breeding system, crossability, hybrid fertility, and homology of chromosome structure (*ibid.*):



The Exigua Cluster contains Exigua (*Stephanomeria exigua* Nutt.), Deanei (*S. exigua* var. *deanei* MacBride), Coronaria (*S. coronaria* Greene), Carotifera (*S. carotifera* Hoover), Paniculata (*S. paniculata* Nutt.), and Macrocarpa, a newly discovered and undescribed taxon.

The Virgata Cluster contains Virgata (*S. virgata* Benth.) and Pleurocarpa (*Ptiloria pleurocarpa* Greene).

The morphological features of the two clusters are combined in an additional diploid species Diegensis (*S. diegensis* Gottlieb, in manuscript), and two different tetraploid taxa. Carotifera also varies morphologically in the direction of Virgata.

The grouping of taxa into two clusters is the result of experimental and observational analyses. Problems of phylogenetic interest develop when we try to analyze the relationships of the morphologically intermediate Diegensis, Carotifera, and the two tetraploids. To illustrate these problems we will make the initial hypothesis that these entities have a hybrid origin between the clusters.

#### THE TETRAPLOIDS

We have a high degree of confidence that the tetraploids are allotetraploids, and I will treat them briefly. Their chromosome number is  $2n = 32$ , an addition of the diploid number of  $2n = 16$  common to all the diploid *Stephanomerias*. They show normal meiosis with bivalent chromosome pairing. They either resemble one or the other diploid cluster or are morphologically intermediate; neither one has any unique morphological features. One of them is found within the zone of geographical overlap of the diploid clusters in central and southern California; the other is found partially within the overlap zone but also to the north of it in the North Coast Ranges and the western foothills of the Sierra Nevada. Both are commonly encountered in disturbed areas along roadsides and freeways and in open habitats such as coastal sand dunes, chaparral openings, oak savannah, and grassy meadows. Triploid  $F_1$  hybrids between different diploids from both clusters and the tetraploids show *Drosera* chromosome pairing with the essential absence of multivalents. Artificial tetraploids synthesized with colchicine from sterile  $F_1$  hybrids between different members of the two clusters had fully restored fertility and exhibited regular meiosis. Consequently, it is possible to satisfy most of the criteria that the tetraploids have arisen as a result of chromosome doubling following interspecific hybridization between species in the Exigua and Virgata Clusters.

#### THE PARENTAL DIPLOID STEPHANOMERIAS

The hypothesis that Diegensis and Carotifera also result from interspecific hybridization between the clusters is more difficult to deal with, and the level of confidence regarding their phylogeny is correspondingly weaker. In order to convey some of the problems in the limited time available I will summarize the relevant evidence and then discuss the possible interpretations.

Virgata and Pleurocarpa are distributed primarily in California (Fig. 1) in oak savannah and chaparral openings; Virgata is also abundant on roadsides and freeway embankments in southern California. The taxa resemble each



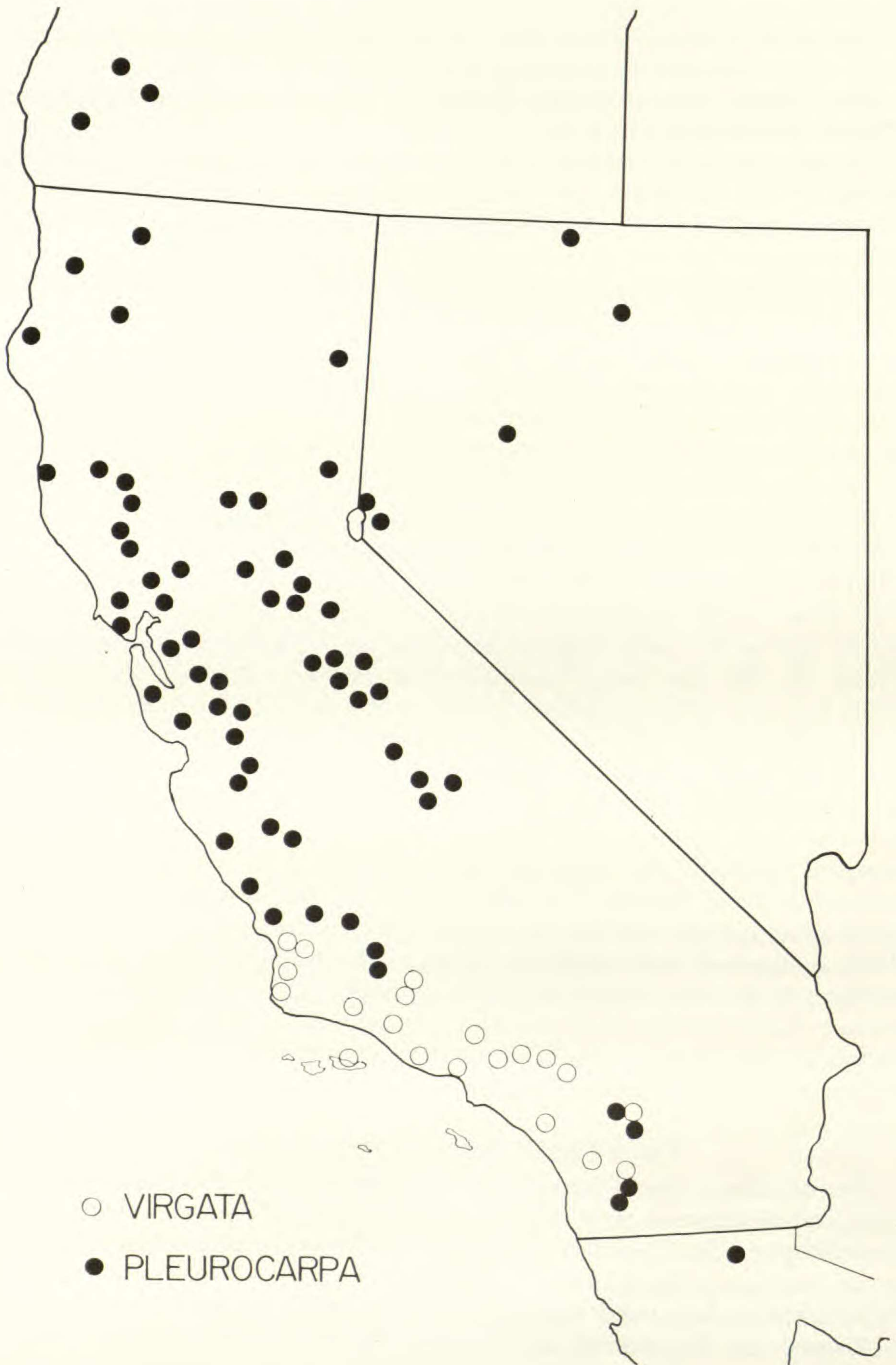


FIGURE 1. Distributions of *Virgata* and *Pleurocarpa*.



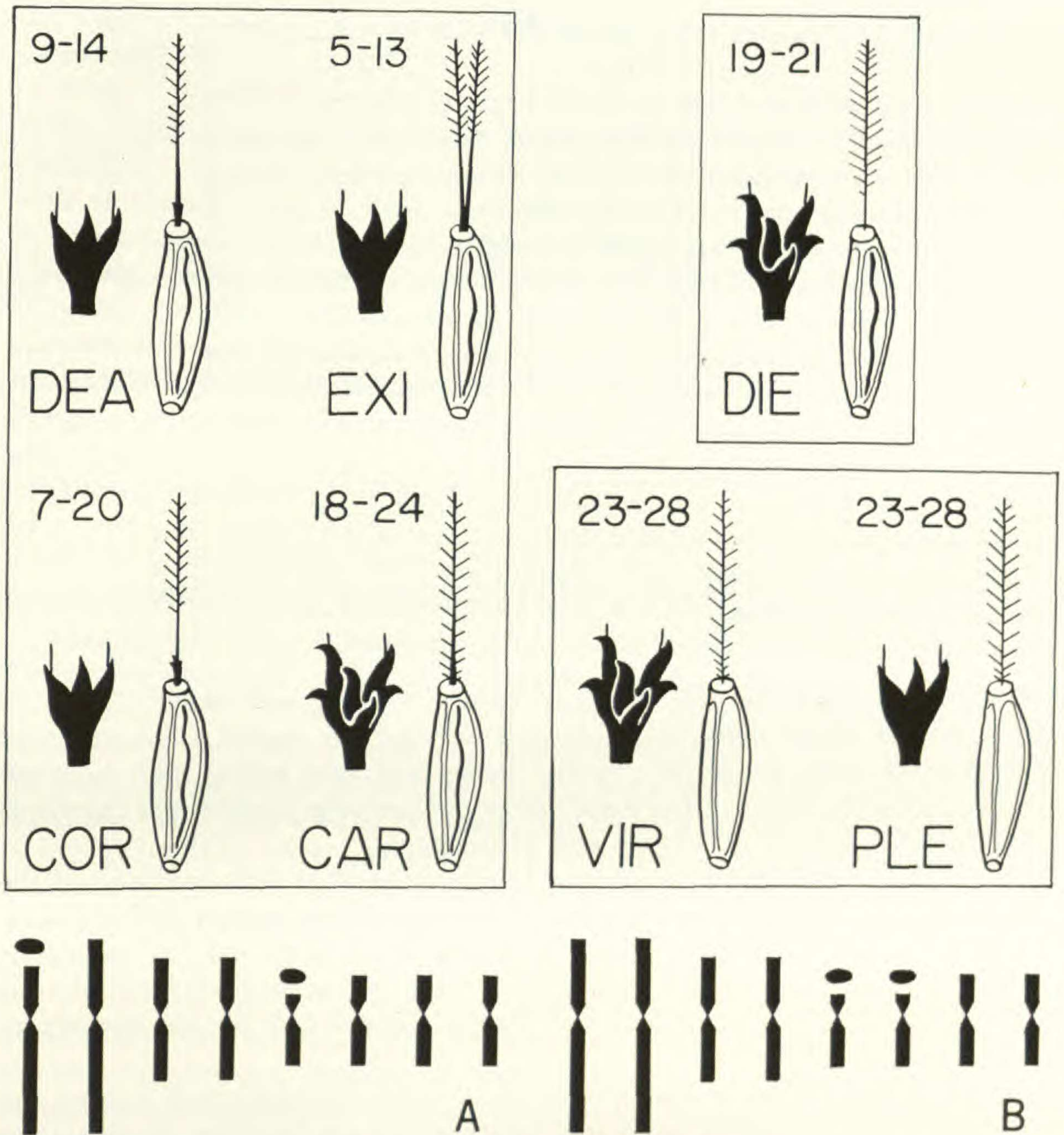


FIGURE 2. Diagram of the karyotypes and morphological characteristics of the achenes, pappus bristles (including number per achene), and basal involucre bracts of different taxa of *Stephanomeria*. Karyotype A is found in *Deanei*, *Exigua*, *Coronaria*, and *Carotifera*. Karyotype B is found in *Diegensis*, *Virgata*, and *Pleurocarpa*.

other in many features of their achenes and pappus bristles, but differ in the condition of the basal involucre bracts which are reflexed in *Virgata* and appressed in *Pleurocarpa* (Fig. 2).  $F_1$  hybrids between them are highly fertile and have mean pollen stainabilities ranging from 78% to 90%, with an overall mean of 84% (Fig. 3). Their chromosomes pair extremely well, and an average of 95% of the PMC's in their  $F_1$  hybrids have eight bivalents.

In the *Exigua* Cluster, *Exigua* and *Coronaria* are widespread in California, the Southwest, and the Great Basin (Fig. 4). *Exigua* is distributed in sandy soils in sagebrush and creosote bush desert communities. *Coronaria* occupies a wide variety of habitats from the maritime Channel Islands to the eastern



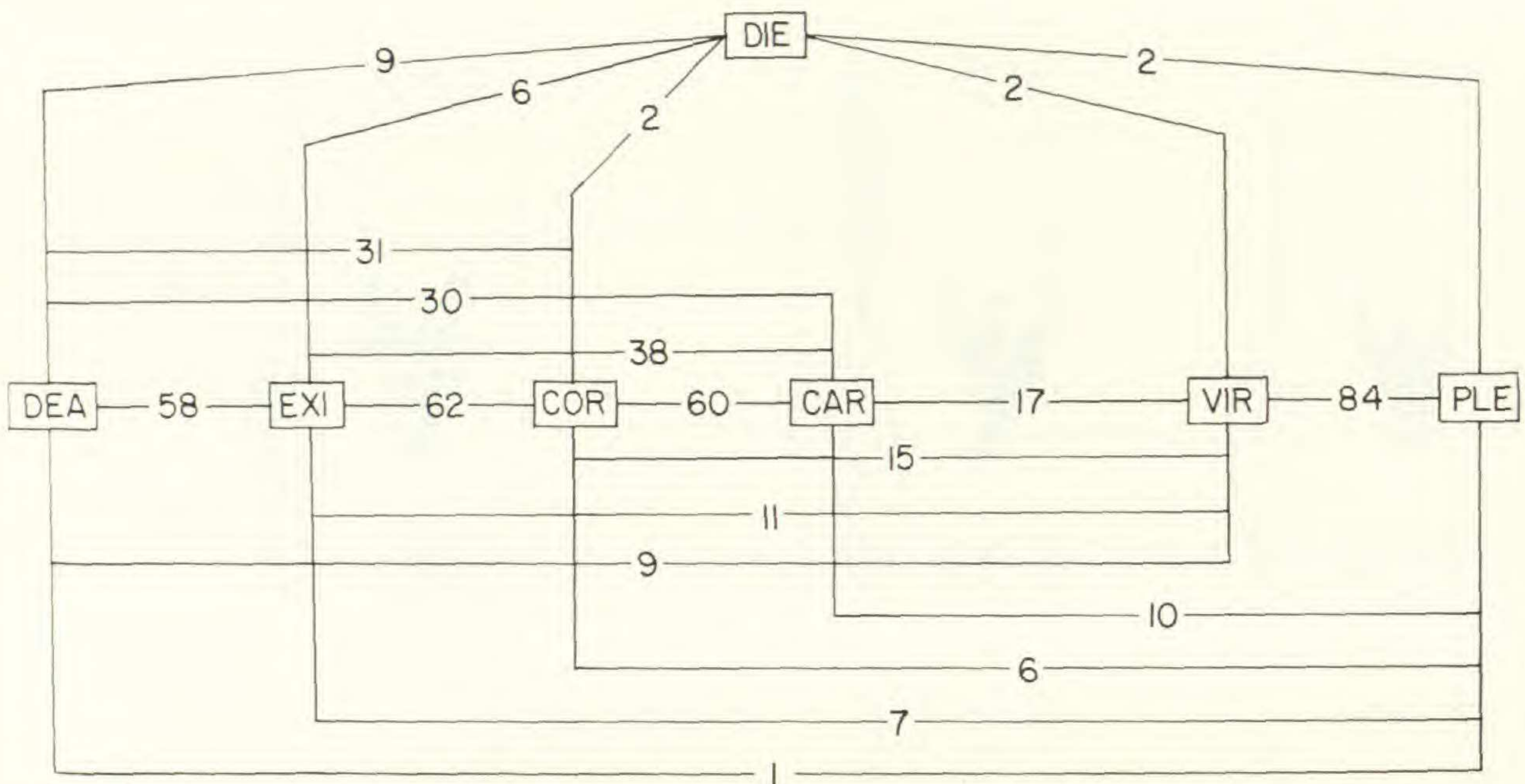


FIGURE 3. Summary of the pollen fertilities (in percent) of  $F_1$  hybrid progenies between different taxa of *Stephanomeria*.

Sierra Nevadas and the cold desert of eastern Oregon and Idaho. Deanei is distributed in sandy fields and chaparral in southern coastal California, and Carotifera on sand dunes and marine shales and arid inland sites in south central California (Fig. 4). The distributions of Coronaria, Deanei, and Carotifera partially overlap those of Virgata and Pleurocarpa.

These four members of the Exigua Cluster as well as both members of the Virgata Cluster have a sporophytic self-incompatibility system and are consequently obligate outcrossers. Paniculata and Macrocarpa are self-compatible and are self-pollinated in the greenhouse. They are most likely derived from different outcrossing members of the Exigua Cluster and do not concern us further here.

The members of the Exigua Cluster share many morphological features but each of them is set off by particular characters or combinations of characters. As a group they exhibit a number of morphological characters which differentiate them from the Virgata Cluster (Fig. 2). The achenes of the Exigua Cluster are grooved longitudinally, and the pappus bristles are less numerous, at least slightly thickened at the base, often connate in groups of two to four, and, with the exception of the fully plumose Carotifera, are 40% to 75% plumose. In contrast, the achenes of the Virgata Cluster lack grooves, and its pappus bristles are more numerous, not thickened, separate at the base, and densely plumose for their entire length. The involucre bracts of Exigua, Deanei, and Coronaria are appressed like those of Pleurocarpa, whereas those of Carotifera are reflexed like those of Virgata. The two clusters also differ morphologically in several other characters of pubescence and inflorescence branchlet length.

The karyotypes of both clusters are similar in relative chromosome lengths and centromere positions. They differ in that in the Exigua Cluster a satellite is borne on the short arm of one long and one short chromosome, whereas in



the *Virgata* Cluster a satellite is found on the short arm of two short chromosomes (Fig. 2).

Forty  $F_1$  hybrid progenies were grown from crosses within the *Exigua* Cluster. Within-taxon progenies were highly fertile with an overall mean of 85% pollen stainability. Between-taxon progenies had means ranging from 30% to 62%, with an overall mean of more than 40% (Fig. 3). Cytogenetic studies of 14 hybrid progenies reveal a high degree of homology in chromosome structure within the cluster. The percentage of PMC's with regular bivalent pairing ranged from 65% to 100%, with an overall mean of 86%. The number of unpaired chromosomes was less than 0.2 per PMC. However, most hybrids had a single quadrivalent suggesting the presence of a reciprocal translocation; and single bridge and fragment configurations, suggestive of a paracentric inversion, were also observed.

Although the members of the *Exigua* Cluster have diverged from one another to a moderate degree both morphologically and reproductively, they remain similar in many respects. They are probably best considered a group of semi-species in which strong discontinuities have not yet developed.

The reproductive relationships of the clusters with each other were tested by growing out twenty different  $F_1$  hybrid progenies. The mean pollen fertility of these hybrids was sharply reduced compared to that of the hybrids within each cluster. Between *Virgata* and different members of the *Exigua* Cluster, the mean hybrid pollen stainability ranged from 9% to 17%; between *Pleurocarpa* and the *Exigua* Cluster the range was 1% to 10% (Fig. 3). The reductions in hybrid fertilities were accompanied by increases in the proportion of PMC's with meiotic abnormalities. For example, for the nine hybrids analyzed cytogenetically, the mean percentage of PMC's with regular bivalent chromosome pairing dropped to 42%. The number of multivalents was high, and each of the  $F_1$  hybrids examined had high percentages of cells with at least one quadrivalent and several of the hybrids had additional and/or larger multivalents. The reduced fertility of these hybrids is most likely the result of differences in chromosome structure between the two clusters. This suggestion is strengthened by the fact that artificially doubling the chromosome number of two between-cluster  $F_1$  hybrids by using colchicine increased their pollen stainability from 16% to 94% and from 6% to 98%. Both artificial tetraploids showed preferential bivalent pairing and no multivalents were observed.

In addition to reductions in hybrid fertility, the two clusters are separated by crossability barriers such that only 5–10% of experimental cross-pollinations between them yielded seed. Within the clusters, 50–75% of the between-taxon cross-pollinations yielded seed. No barriers reducing crossability were detected between populations of the same taxon.

A number of between-cluster natural hybrids have been found where populations of the clusters are sympatric. Like the experimental hybrids, these individuals are vigorous and retain partial fertility. Consequently, they satisfy the criterion that the putative parents can hybridize in nature to produce at least partially fertile offspring. The opportunity for sympatry is high in



California where their distributions widely overlap and where they occupy habitats which are often juxtaposed.

The differences between the Exigua and Virgata Clusters in morphology, karyotype, chromosomal structural arrangement, crossability, and hybrid fertility are believed to reflect a fundamental phylogenetic divergence within the annual *Stephanomerias*. The hypothesis of hybridity of the morphologically intermediate entities requires that the parental taxa be phylogenetically older than the derivative taxa. Although there is no fossil evidence, the great age of the clusters is attested to by their wide distributions, and broad ecological amplitudes. In addition, they are the progenitors of two different tetraploid entities. The Exigua Cluster contains morphologically and reproductively divergent taxa and also includes two derivative self-compatible entities, one of which (*Paniculata*) is widespread.

#### THE PUTATIVE HYBRID DIPLOID STEPHANOMERIAS

Having reviewed the characteristics and relationships of the two clusters, we can examine more closely the evidence that *Carotifera* and *Diegensis* result from their hybridization.

Let us look first at *Carotifera*. The available evidence satisfies many of the criteria for its having a hybrid origin. In overall morphology, karyotype, and chromosomal homology, it resembles other members of the Exigua Cluster. However, in several morphological features, it varies in the direction of *Virgata*. For example, it has reflexed involucre bracts and fully plumose pappus bristles, and it has an intermediate number of bristles and an intermediate degree of bristle base thickening. Despite the geographically restricted distribution of *Carotifera*, it shows a high degree of inter-population morphological variability in a number of characters such as ligule color, floret number and length, rosette leaf width, and degree of bristle base thickening.

The bristle base character is particularly interesting, because it divides *Carotifera* into two groups of populations. Inland populations have markedly thickened bases like other taxa in the cluster, while coastal populations have very slightly thickened bases, varying toward those of *Virgata*. Colinear with this morphological difference, the inland populations are reproductively closer to *Coronaria* than are the coastal populations. Hybrids between the inland populations and *Coronaria* are 75% fertile, whereas those between the coastal populations and *Coronaria* are only 45% fertile. The inland and coastal populations are fully interfertile. This difference in reproductive compatibility is not shown towards the geographically more remote *Exigua* and *Deanei*.

*Carotifera* is distributed where other members of the Exigua Cluster and *Virgata* and *Pleurocarpa* are sympatric in the South Coast Ranges. Hybrids between the two clusters have been found in this region. Inland populations of *Carotifera* are also presently sympatric in several localities with both *Virgata* and *Pleurocarpa*, and hybrid individuals have been observed in these sites. Seeds taken from a natural hybrid between the inland form of *Carotifera* and *Pleurocarpa* were grown in a progeny test. Three seedlings matured. They were similar morphologically to *Carotifera* in some characters and intermediate in



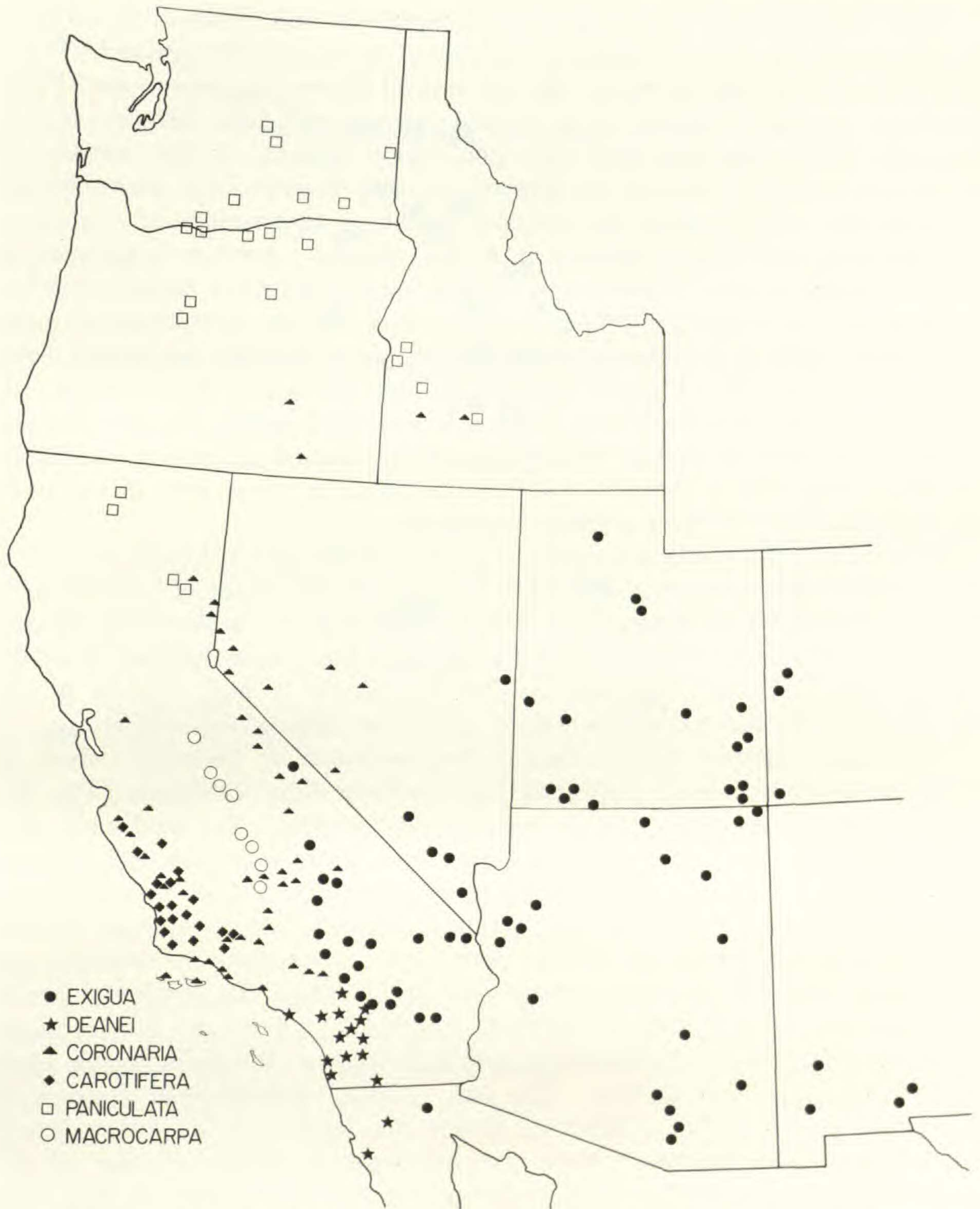


FIGURE 4. Distributions of *Exigua*, *Cornaria*, *Deanei*, *Carotifera*, *Paniculata*, and *Macrocarpa*.

others. Their pollen stainabilities were 23%, 23%, and 55%, whereas the mother plant had only 11% stainable pollen. The seedlings were apparently backcrosses to *Carotifera*, and the increase in their fertility indicates that selection can be expected to operate rapidly for this trait.

The ecological preferences of *Carotifera* are open, temporary, and pioneer habitats. Coastal populations are found on sand dunes and shales; the inland populations favor sandy sites in grasslands and oak savannah in the arid inner South Coast Ranges.



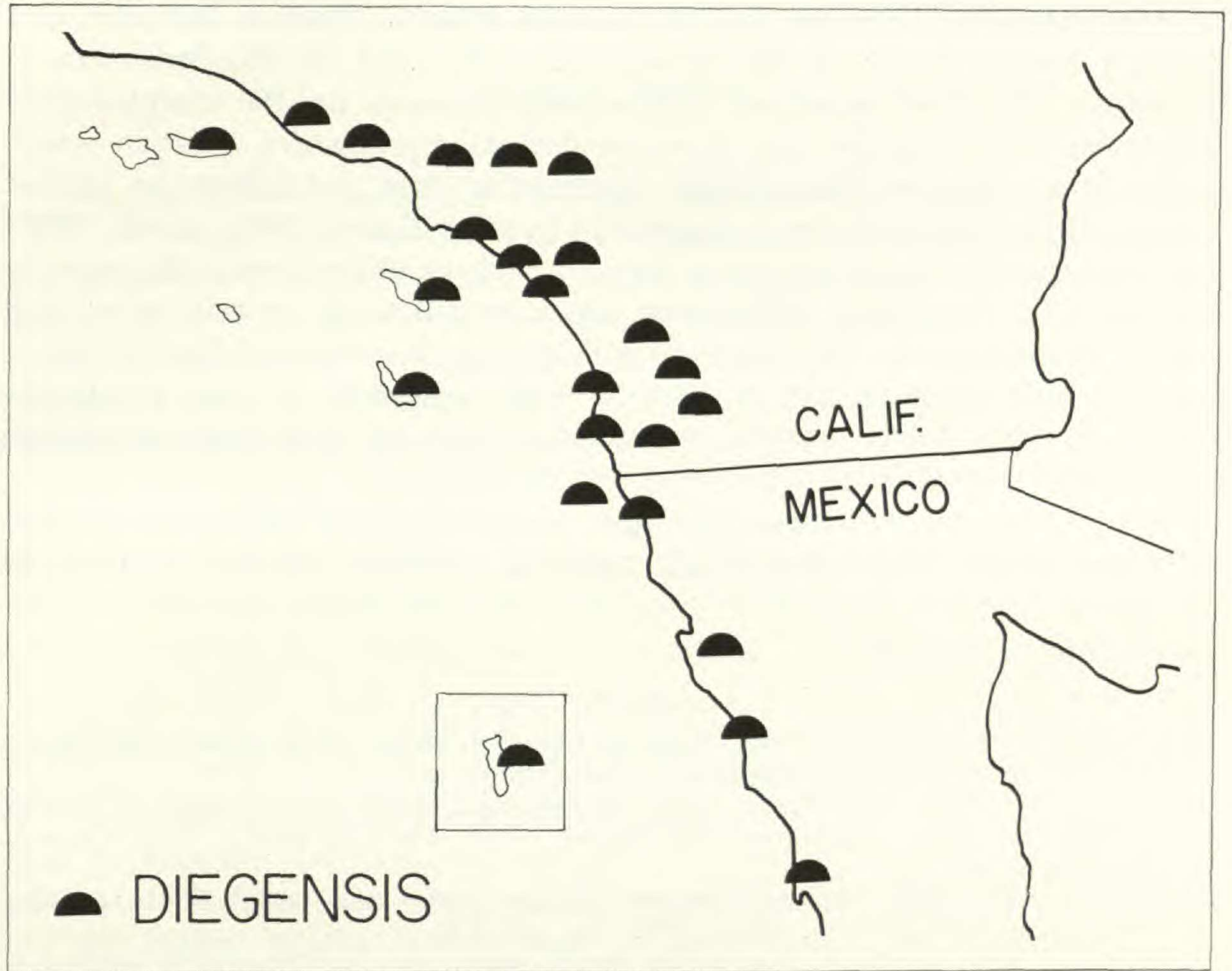
With the exception of an experimental synthesis and biochemical studies, the criteria for the hybrid origin of *Carotifera* are reasonably satisfied. However, it would be well to try to rule out several alternative hypotheses. First, *Carotifera* may be a remnant of an ancient entity from which the two clusters diverged. This suggestion does not seem likely because of the recency of its habitats and preferences for temporary sites, features not generally associated with ancient relics. In addition, the close relationships of *Carotifera* to *Coronaria* and *Exigua* suggest it is not ancient. Another hypothesis is that the resemblance of *Carotifera* to *Virgata* may result from mutation within the gene pool of *Carotifera* followed by selection for the morphological traits in question. This is a difficult hypothesis to prove because we would have to know the number of genes involved in the characters, their linkage relationships, their regulation, and their selective advantages. In the absence of this information as well as information on the amount of genetic variability for these characters in the gene pool of *Carotifera* at some time in the past, we have no reason to favor such an hypothesis.

In summary, the evidence we have suggests that the morphological characters of *Carotifera* that cause it to resemble *Virgata* are the result of hybridization between *Coronaria* or *Exigua* and *Virgata* followed by backcrossing to the *Exigua* Cluster parent accompanied by selection for a new balance of adaptation. The level of confidence in this hypothesis is high, but it is not as high as with the tetraploids where additional criteria are satisfied.

The other putative hybrid diploid *Stephanomeria* is *Diegensis* which is found in southern coastal California and northern Baja California (Fig. 5). It also occupies pioneer habitats such as old clearings, the landward sides of coastal sand dunes, chaparral openings, and sandy roadsides and freeway embankments. *Diegensis* has a different combination of the morphological characters of the clusters. It has grooved achenes, but its pappus bristles are separate, not thickened at the base, and intermediate in number and plumosity (Fig. 2). In the southern part of its range, the involucre bracts are strongly reflexed, but in northern populations they are less reflexed. *Diegensis* also combines pubescence and inflorescence branchlet length characteristics of the two clusters. The only unique morphological features of *Diegensis* are its higher number of florets per head and the longer lateral pinnae along the pappus bristles. Its karyotype is similar to that of the *Virgata* Cluster in having satellites borne on the short arms of two short chromosomes (Fig. 2).

*Diegensis* is the most reproductively isolated taxon of all the outcrossing diploid *Stephanomerias*. Twelve different  $F_1$  hybrid progenies between it and the other taxa had mean pollen stainabilities ranging from 2% to 9% (Fig. 3). The reduction in fertility of these hybrids is accounted for by the finding that the chromosomes of *Diegensis* are extensively repatterned from those of the other taxa. In the five different  $F_1$  hybrids studied cytogenetically, the percentages of PMC's with regular bivalent pairing ranged from 1% to 29%, and the number of unpaired chromosomes was substantial, ranging from 2.3 to 6.6 per PMC. Multivalents and bridge and fragment configurations were



FIGURE 5. Distribution of *Diegensis*.

common. Not only is hybrid fertility reduced, but crossability barriers are also present since it was difficult to obtain hybrids with *Diegensis* as a parent.

The strong reproductive isolation of *Diegensis* increases the complexity of evaluating its phylogenetic relationships. Three hypotheses can be suggested. First, *Diegensis* may be an ancient relic and is a morphological composite of the two clusters, because they differentiated from it or from a similar taxon. The problem with this hypothesis is that it cannot explain why members of the two clusters diverged in morphology, karyotypes, and ecological adaptations, and yet failed to diverge reproductively to the same extent, remaining more compatible with each other than with their supposed progenitor. Also it seems unlikely that *Diegensis* is an ancient relic, because it is found on temporary and often disturbed sites. A second hypothesis assumes that the *Exigua* Cluster, the *Virgata* Cluster, and *Diegensis* are each derived from a different perennial *Stephanomeria* and consequently show different degrees of reproductive isolation. This hypothesis requires that the perennial species that gave rise to *Diegensis* was morphologically intermediate, which simply removes the problem of explaining the intermediacy to an earlier time. Also, the extant perennial species of *Stephanomeria* are distinct from each other, and none of them exhibit morphological intermediacy. The third hypothesis is that *Diegensis* also results



from hybridization between the Exigua and Virgata Clusters but one that occurred independently of the hybridization that led to the formation of Carotifera. The third hypothesis satisfactorily accounts for the morphological intermediacy of Diegensis and presumes that its reproductive isolation which results from extensive chromosomal repatterning does not reflect an ancient lineage. It has frequently been suggested (Lewis & Raven, 1958; Lewis, 1966) that hybrid sterility in annual plants often results from differences in chromosome structure and that these differences can accumulate in a relatively small number of generations. The level of confidence in the inference that Diegensis has a hybrid origin is not as high as with Carotifera or the tetraploids. This is because fewer criteria are satisfied and an additional assumption is necessary to explain its sharp reproductive isolation.

In summary, *Stephanomeria* illustrates the problems of attempting to deal with past events. Depending on the available evidence and the adequacy of the criteria satisfied, the best we can do is establish levels of confidence for the inferences we make.

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